

Recent Research in Science and Technology 2009, 1(4): 180–183
ISSN: 2076-5061
www.recent-science.com



MEDICAL MICROBIOLOGY

RESISTOTYPING OF *CANDIDA ALBICANS* ISOLATED FROM SPUTUM SAMPLES OF PATIENTS ATTENDING TB CLINICS IN AND AROUND CHIDAMBARAM

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Abstract

Resistotyping was first developed for strain delineation, pathogenesis, or epidemiological studies. This depends on the ability of the strains to grow on agar plates containing various dilutions of toxic chemicals. There are many resistotyping methods and modified techniques were used by the authors to carry out the typing of clinical isolates of *Candida*. We performed resistotyping of 350 *Candida albicans* (isolated from the sputum of TB clinic attendees) with modified technique and could able to obtained four (04) different resistotypes among–B–F– resistotypes was found to be the predominant *Candida albicans* resistotypes we recorded. From our one year study period from June 2007 to May 2008, we concluded that the *Candida albicans* resistotypes –B–F– was the most commonly found resistotype in the specified areas of Cudalore district of Tamilnadu, India.

Key Words: Resistotyping; *Candida albicans*; TB clinic attendees.

Introduction

Resistogram typing was first developed for bacteria in early 1970s. Resistogram methods can be used for strain delineation, pathogenesis, or epidemiological studies. This depends on the ability of the strains to grow on agar plates containing various dilutions of toxic chemicals. Bacterial species are delineated to strains based on their resistance or susceptibility to selected organic or inorganic compounds or both. In 1974 Warnock et al developed a Resistogram typing method for distinguishing strains of *Candida albicans* (1). Originally 6 chemicals malachite green, boric acid, sodium arsenate, copper sulphate, acrylamide and 4-chlororesorcinol were tested at 6 different concentrations. And many investigations on resistotyping was carried out and found out the resistance pattern of the different strains of *Candida* to different chemicals (2,3,4). There are many resistotyping methods and modified techniques were used by the authors to carry out the typing of clinical isolates of *Candida*.

Many publications are available on the resistance pattern of certain *Candida albicans* biotypes/resistotypes from oral cavity and other sites (5-9). It has been found out that there may be geographical differences among certain resistotypes isolates frequencies. Only limited

data available for the resistotyping of *Candida albicans* especially from India. There seems to be absence of the study related to the *Candida albicans* resistotypes from southern part of Tamilnadu, India. The intension of the present study is to isolate the *Candida albicans* strains present in the sputum of the patients in and around Chidambaram and to study the resistotyping pattern to certain chemicals and find out prevalent *Candida albicans* strains colonizing/ associated with respiratory tract infection especially with the patients attending TB clinics.

Materials and Methods

Subjects included

A total of 2800 patients with signs and symptoms of respiratory tract infection attended the outpatient departments at TB clinics at Rajah Muthiah Medical College and Hospital (RMMC&H), Chidambaram and various private nursing homes in and around Chidambaram were included in this study. The study was carried out for one year period from June 2007 to May 2008.

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Selection criteria

The patients with signs and symptoms of respiratory tract infections attended TB clinics were included in this study. Prior to specimen collection patients were enquired about the antibiotic treatment if they were undergoing or underwent.

Specimens Collected

After getting patient's concern, three consecutive days early morning sputum was collected. The patients were given adequate instructions to gargle for 4 to 5 times with the tap water and collect the sputum samples after inducing deep cough. Totally from 2800 clinic attendees the sputum specimens were collected. The collected samples were safely transported to the division of mycology laboratory, department of microbiology for further proceedings.

Direct microscopic examination

10%KOH mount

The sputum samples were screened for the yeast cells with or without pseudohyphae (10).

Gram's staining

Uniform smears either from the purulent, mucopurulent or blood stained portion of the sputum was prepared and subjected to Gram stain and examined (10).

Candida Culture

The sputum collected from the TB clinic attendees were subjected for *Candida* isolation and inoculated on one set of Sabouraud dextrose agar (SDA) plates with antibiotics (Chloramphenicol, 50mg/1000ml). One plate incubated at 37°C and other at 25-30°C (BOD incubator) for one week. The typical creamy, smooth pasty colonies with yeasty odour were noted and recorded.

Candida albicans Identification

The isolated *Candida* species were subjected to the germ tube test, Chlamydospore formation and growth on Chrome agar (Hi-Media) and the speciation was carried out. The *Candida albicans* species alone selected and subjected to resistotyping.

Resisto typing method

The resistotyping method of Medcraft's (12) modification of McCreight and Warnock's (13) method was used in this study. This depends on the ability of strains to grow on agar plates containing various dilutions of toxic chemicals. Table1 shows the chemicals used, their code and concentrations.

Table 1. Chemicals used in resistotyping and the code used

No	Chemical compound	Letter code	Conc. of stock solution (g/l)	Volume added to resistogram plates (ml/20ml agar)			
1	Sodium selenite	A	20	0.5	0.6	0.7	0.8
2	Boric acid	B	20	1.2	1.3	1.4	1.5
3	Cetrimide	C	20	0.05	0.1	0.15	0.2
4	Sodium periodate	D	20	0.3	0.4	0.5	0.6
5	Malachite green	F	0.02	0.2	0.3	0.4	0.5
6	Copper sulphate	G	20	1.0	1.2	1.4	1.6

The selected chemicals with specified concentrations prepared as stock solution and the different volumes of the chemicals added to the SDA are presented in Table 1. Then each plate were divided in to 8 sections (Figure1) and different *Candida albicans* isolates, isolated from the sputum of the TB clinic attendees were inoculated and incubated at 37°C for 48 hrs and the results was recorded. The candida resistotypes were recorded when there was no growth appears on the streak.

Results

The *Candida albicans* isolated from the sputum of the TB clinic attendees subjected to the resistogram against six chemicals and we could able to obtained 4 different resistotypes (Table 2 & Figure 2)

Table 2. Results of resistogram typing

Candida	Resistotypes	No. of strains (%)
C.albicans n=350	—B—F—	175(50.00)
	AB—F—	60 (17)
	—B—F—	58 (17)
	—F—F—	57 (16)

Briefly each chemical is designated by a letter code as shown in the Table1. Thus, for example, the resistogram "AB—F—" signifies that the tested strain was resistant to sodium selenite(A), Boric acid(B) and Malachite green(F), but sensitive to Cetrimide(C), Sodium periodate(D) and Copper sulphate(G).

The *Candida albicans* resistotypes identified from our study was designated as —B—F—, AB—F—, —B—F— and —F—F—. Among 350 *Candida albicans* isolates 50% of the *C.albicans* belongs to the resistotype category —B—F—, followed by the resistotypes AB—F— (17%), —B—F— (17%), and —F—F—(16%)(Table 2 & Figure 2).

Discussion

Candida is one of the fungi, yeast like organism, which infects both immunocompetant and immunosuppressive hosts. Even though increasing incidence of other species of candida has been reported recently, *Candida albicans* still remains the most commonly isolated yeast from the clinical specimens (11). It was quite interesting to note that the significant

presence percentage (23%) of *Candida* species in respiratory specimens, in which *Candida albicans* alone represented 53% and gaining the support of the above statement (11).

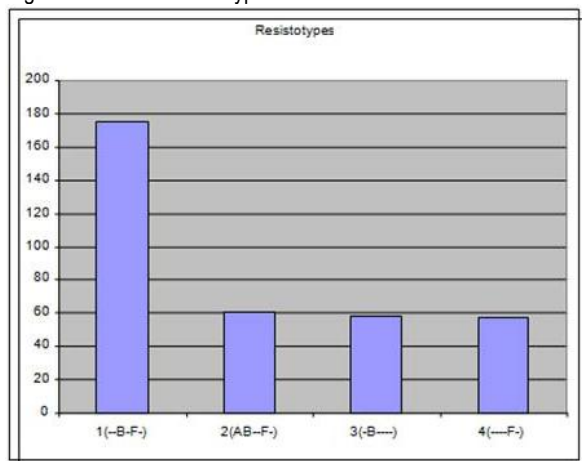
The adopted method of resistotyping in our study divided the *Candida albicans* species in to four (4) different resistotypes (Table 2) (Figure 2). Furtherly we could able to note that among the four resistotypes, —B— —F— (50%) was found to be the predominant one, which was isolated from the sputum of the TB clinic attendees followed by other resistotypes AB— —F— (17%), —B— — — (17%) and — — — —F— (16%) respectively.

Figure 1: Growth of *Candida albicans* on different concentrations of SDA with Malachite green



Some authors included Silver nitrate as one of the chemicals in their resistotyping study, but with reference to Khatib et al 1995 the addition of the two new chemicals to the resistogram typing set has increased the discrimination of the method (14). In our study the chemical silver nitrate was not used as it has poor discrimination and reproducibility which was already mentioned by Hunter and Fraser 1987.

Figure 2: Different resistotypes



The studies related to the virulence factors of *Candida* such as Toxins, Enzymes, Adhesin Complement receptors and phenotypic switching had been described and the comparative studies with these factors and the antifungal resistance have also been carried out. Pathogenicity of the *Candida* strains was correlated with certain resistotypes of *Candida* by Giovanni et al in 2005 and noted that the resistotype - - C- - was found to be the most common isolate, and was the most pathogenic. But Hunter et al 1987 mentioned that the resistotypes - - C- - was found rarely in their study.

During our study period, there seems to be the total absence of this particular *Candida albicans* (- - C- -) and its presence in the sputum of the TB clinic attendees. It has been noted by Hunter et al 1987 that there was no association of any particular type of *Candida albicans* with vaginal Candidiasis (15). In our study we could able to record there was no association of particular *Candida albicans* resistotypes either with pulmonary TB positive or negative cases. All four different *Candida albicans* resistotypes were uniformly found in the respiratory tract of both TB positive and TB negative cases. Hunter and Fraser found that the three resistogram types, A—C—F—, ABC—F— and A— — —F— were the major ones among various biotypes observed from the fecal and vaginal isolates of *Candida albicans*. These resistotypes were not found in the pulmonary isolates of *Candida albicans*. In our present study “—B— —F—” was the predominant (50%) resistogram type we recorded.

From our one year prospective study we can conclude that 12.5% of *Candida albicans* were isolated by conventional mycological methods from the sputum specimens of the TB clinic attendees of RMMCH, and various hospitals in and around Chidambaram, Tamilnadu, India. These *Candida albicans* isolates were subjected to biotyping by resistogram method susceptibility to sodium selenite, boric acid, cetrimide, sodium periodate, malachite green and copper sulphate. And four different resistotypes we could able to obtain and “—B— —F—” (50%) is the most commonly found resistotype in the specified areas of Cudallore district of Tamilnadu. And further research is essential to confirm our statement and it was noted that the resistotyping method shows that no particular resistotype was associated in a particular age group or sex.

Candida albicans are the opportunistic pathogens involved in an increasing number of infections of patients with immunodeficiency. But their subspecies discrimination is necessary for epidemiological investigations and studies on the source of infection, transmission of strains and outbreaks of systemic candidiasis. Different typing methods at the phenotypic and genotypic level serve the purpose which includes

various molecular technologies. Unfortunately, most of these typing methods, especially genotypic methods, are expensive and technically demanding. But this resistotyping method we used in our study is an easy one to perform and suggested as applicable for *Candida albicans* or any other *Candida* species for the strain discrimination when more number of strains to be discriminated. To justify our study result and or to criticize, the need of further extensive study in the same field in same geographic areas is essential and welcome.

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